Ultrafast Synaptic Endocytosis Cycles to the Center Stage

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Upon synaptic vesicle fusion, the neuronal membrane surface expands. Clathrin-mediated endocytosis compensates for this expansion but is a slow process. Recent studies in *eLife* and *Nature* identify an ultrafast mode of endocytosis, occurring in the space between active and periactive zones, that compensates for membrane addition during vesicle fusion.

Communication within the nervous system is mediated by synaptic vesicles that fuse with the presynaptic membrane to release neurotransmitters. In order to maintain proper brain function, literally billions of synaptic vesicles are continuously utilized. Given that axonal transport is too slow to supply enough membrane material to maintain proper levels of neurotransmitter release, these vesicles undergo local cycles of fusion and endocytosis at the synapse. Hence, while it is evident that efficient vesicle retrieval at the synapse is essential for the nervous system to function, the paths by which presynaptic membrane is recycled following stimulation are not fully elucidated.

Two studies from the Jorgensen group, published in *eLife* and *Nature*, have now identified an evolutionary conserved mode of membrane retrieval that occurs following mild stimulation and is dependent on actin and dynamin (Watanabe et al., 2013a; Watanabe et al., 2013b). In a technical stride, Watanabe and colleagues managed to image synaptic contacts in *C. elegans* neurons (Watanabe et al., 2013a) and in mouse neurons (Watanabe et al., 2013b), as early as tens of milliseconds following stimulation, at electron microscopy resolution. By analyzing the images taken at defined, increasing time intervals following a single stimulus, they noticed that blebs of membrane form and are internalized immediately adjacent to the presynaptic dense body where synaptic vesicles had fused, as to "instant compensate" for the membrane that was added. How these membrane invaginations are then resolved into normal-sized synaptic vesicles remains to be elucidated.

Different modes of vesicle recycling have been proposed to coexist, including clathrin-mediated endocytosis (CME), kiss-and-run, and bulk endocytosis (Figure 1), but they are all different from the mechanism revealed by Watanabe et al. The best-studied mechanism is CME (Heuser and Reese, 1973), a version of which is also being utilized by numerous membrane-bound receptors and signaling molecules. An elaborate machinery that is active in the periactive zone (Figure 1) is involved in sorting proteins and lipids into small membrane patches. These patches are then molded into deeply invaginated pits that are pinched off of the membrane to create new synaptic vesicles. This process takes time: creating a new vesicle via CME requires several seconds (McMahon and Boucrot, 2011) and leaves the outstanding question of what happens to the membrane that was added following vesicle fusion but before retrieval.

Both bulk endocytosis and kiss-and-run do ensure instant membrane retrieval. During intense bouts of stimulation, large blebs of membrane pinch off from the presynaptic membrane and this process of bulk endocytosis seems to be inhibited by clathrin (Kasprowicz et al., 2008). Conversely, during mild stimulation, in a process called kiss-and-run (Alabi and Tsien, 2013; Ceccarelli et al., 1972), a synaptic vesicle that is in the process of fusion is pinched off the membrane within a time frame of only seconds to rejoin the active zone-bound synaptic vesicle pool. In kiss-and-run, there is no need to sort proteins or lipids because the vesicle remains intact. While this latter mechanism is elegant in design, its existence has remained controversial. The identification of the exact molecular mechanism that mediates this form of recycling has trailed behind, and, in addition, direct visualization of "kissing vesicles" has not been achieved. The methodology used by Watanabe et al. (2013a, 2013b) has a time frame that is well within the resolution of kiss-and-run and is ideally suited to observe such vesicles attached by a fusion pore close to the dense body. However, under the conditions the authors used, all the exocytic vesicles observed had broader openings, as if they were in the process of full collapse, suggesting that at least in this context, kiss-and-run is not an important route of vesicle recycling.

While bulk endocytosis and CME are prevalent in the periactive zones, the membrane blebs internalized via ultrafast endocytosis observed by Watanabe et al. (2013a, 2013b) appear directly adjacent to the dense bodies. A similar observation under very different conditions had been made in *Drosophila* photoreceptors (Koenig and Ikeda, 1996). In that case, however, vesicle fusion and membrane recycling were uncoupled by using a temperature-sensitive dynamin mutant that blocked endocytosis at the nonpermissive temperature, thereby causing vesicle depletion when the neurons were stimulated. Return to the permissive temperature showed membrane invaginations directly adjacent to the presynaptic densities, similar to where Watanabe et al. (2013a, 2013b) observed ultrafast endocytosis. This spatial restriction is peculiar and interesting, as it may signify the existence of specialized membrane plates that prevent ultrafast membrane

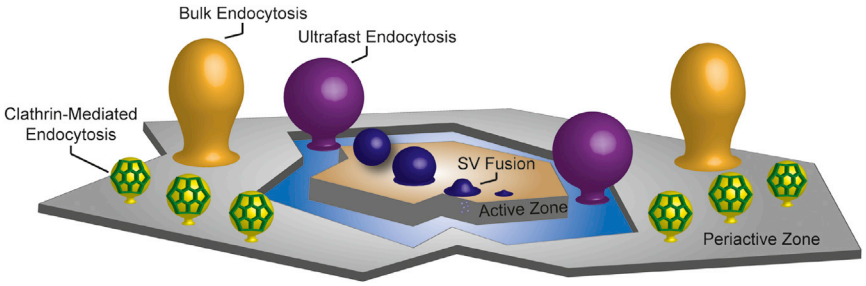

Figure 1. Spatial Organization of Vesicle Cycling at the Synapse
At the active zone, synaptic vesicles undergo full-collapse fusion with the membrane to release neurotransmitters into the synaptic cleft. Directly adjacent to the active zone, a proposed interstitial zone is the location of the newly identified ultrafast endocytosis, which can quickly replenish the membrane surface area during intense stimulation. The interstitial zone is hypothesized to be less densely packed with proteins and cytoskeletal elements required for other forms of synaptic endo- and exocytosis (not shown), thereby allowing large invaginations of membrane to form. Surrounding the interstitial zone is the periactive zone, where other endocytic processes such as bulk endocytosis and clathrin-mediated endocytosis aid in membrane retrieval, synaptic vesicle regeneration, and transmembrane receptor internalization.

blebbing. The dense body-plate region is packed with proteins specializing in vesicle fusion, while the periactive zone plate and associated cytoskeleton specialize in CME or bulk retrieval (Figure 1). At the crevice between the plates, where the membrane may be less rigid, ultrafast membrane invaginations can form (Figure 1). Such a spatial organization of the presynaptic membrane is in line with immunohistochemical and immunoelectron microscopic studies of endocytic markers (Jiao et al., 2010) and may have been set in place to optimally organize membrane recycling at synapses.

An extensive number of studies have highlighted the importance of CME under conditions of repeated and more intense stimulation (Kasprowicz et al., 2008). When neurons defective in CME are presented with only mild stimulation, neurotransmitter release is not much affected. Hence, during mild stimulation, CME may not be absolutely required to recycle the membrane, and ultrafast endocytosis prevails. This division of labor is interesting in light of mechanisms of synaptic plasticity that probably kick in under conditions of stronger stimulation rather than single action potentials. Vesicles that recycle by CME may enter an endosomal compartment, and at this site, proteins can be sorted and reshuffled to optimize protein and lipid content on each synaptic vesicle (Uytterhoeven et al., 2011). This sorting may occur much less often in an ultrafast pathway, where little time is at hand. Hence, ultrafast recycling may be a default mechanism to maintain the surface area following vesicle fusion, while CME (and bulk endocytosis) may impose different layers of regulation of synaptic vesicle function when neurons are being stimulated intensely.

The discovery by Watanabe et al. (2013a, 2013b) reveals a mode of vesicle recycling that is prevalent during physiological, mild stimulation. While actin and dynamin are needed for the process, it will now be interesting to assess the other molecular components that are required to create these invaginations and whether this machinery is different from the one needed for bulk membrane retrieval that ensues during very strong stimulation. It will also be exciting to learn what machinery is involved in resolving the membrane invaginations into small synaptic vesicles and how presynaptic membrane plates and their components determine what type of membrane recycling can occur. Finally, it will be interesting to learn the functional consequences if this ultrafast endocytic pathway is specifically blocked, as well as whether this mechanism of membrane recycling has any bearing in neuronal disease.

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